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Ontogeny of the Sporangia of *Sphaeropteris cooperi*

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ABSTRACT.—The ontogeny of the sporangia of *Sphaeropteris cooperi* was studied using cleared whole mounts of sporangia in different stages as well as sori embedded in paraffin and sectioned. The sporangia develop from a single superficial primordial cell that becomes divided into five initials or "segments." Segment 0, located at the level of the surface receptacular cells, does not become subdivided and does not contribute further to the structure of the mature sporangium. Segments I, II, III and IV each become subdivided through a series of divisions to produce the mature sporangia. The four-rowed sporangial stalks are formed from Segment I and part of Segment II, and the capsules develop from a part of Segment II and Segments III and IV. The annulus develops in Segments II and IV. The developmental pattern of the sporangia of *Sphaeropteris cooperi* is compared to that of the sporangia of higher leptsoporangiate ferns.

The most familiar and most frequently illustrated leptosporangia are those of the higher leptosporangiate ferns. The development of the sporangia of the higher leptosporangiate ferns was described in a series of papers (Wilson,1958a, b, 1960) and is now well understood. In the sporangia of the advanced leptosporangiate ferns, as illustrated by species in the Polypodiaceae, Grammitidaceae, and Vittariaceae, it was shown that the stalk and the capsule of the leptosporangium develops from a single epidermal primordial cell that becomes divided into five initials or "segments," rather than from the activity of an apical cell. Each one of these "segments" in turn divides, through a series of divisions to produce the mature sporangium. Segment 0 contributes only to the formation of the stalk; Segment I to a portion of the stalk and part of the proximal face of the capsule; Segment II to the stomial region, the stalk, and to the proximal and distal faces of the capsule; and Segments III and IV to the rest of the annulus and to both the proximal and distal faces of the capsule. Although the stalk may be one-, two- or three-rowed at its base, the capsule is always subtended by a three-rowed stalk. The one-rowed stalk results directly from the horizontal orientation of the first division of the sporangial initial, whereas the two- and three-rowed stalks depend on the orientation of both the first division and also the division that produces Segment I.

A review of the history of our knowledge of the nature of the leptosporangium and its development was published in the introduction to the study of the ontogeny of the sporangia of *Phlebodium aureum* (L.) J. Sm. (Wilson, 1958a). Recent descriptions of sporangial development continue to reproduce the erroneous pattern apparently originated by Campbell (1905) that the sporangial initial produces a three-sided apical cell that cuts off several basal cells to form the stalk until a transverse division stops its activity by cutting off the cap cell. Other accounts are unclear, incomplete and often incorrect. (see Gifford and Foster, 1989; Bold et.al., 1987; Holttum et. al., 1970). No detailed ontogenetic studies have been published since the appearance of the paper on

the sporangium of *Anarthropteris lanceolata* (Hook. f.) Pic. Serm. (as *A. dictyopteris* (Mett.) Copel.) (Wilson, 1960).

As pointed out in a study of mature sporangia of species of the Polypodiaceae, Grammitidaceae, and Vittariaceae (Wilson, 1959), the cell arrangement in the sporangia reflect the ontogeny of these structures and, with but few exceptions, there is no reason to doubt that the development of the capsules follows the pattern of those of *Phlebodium* (Wilson, 1958a), *Xiphopteris* and *Pyrrosia* (Wilson, 1958b), and *Anarthropteris* (Wilson, 1960). Edwards (1996) expanded the examination of the structure of mature sporangia by initiating a survey of the cellular structure of the capsules of more than 110 species in 20 families. Three of these species were illustrated in his published abstract.

Sporangia with four-rowed stalks, however, are known in several fern genera including *Dipteris, Cheiropleuria,* and members of the Cyatheaceae. Wilson (1959) pointed out that it was not possible to homologize the sporangial faces of *Dipteris* and *Cheiropleuria* with those of the higher leptosporangiate ferns. The known developmental patterns cannot give rise to a four-rowed stalk. Bower (1915) wrote that in *Cheiropleuria* sporangia with four-rowed stalks, the segmentation of the young sporangium, "Appears to show a regular cleavage of the segments in two opposite rows," and the "Subdivision of the two rows of segments of the stalk by walls in the plane of the drawings has given rise to the four rows of cells of the stalk, as seen in later stages." This suggests a distinctly different developmental pattern in these sporangia than is known. The only studies of the development of sporangia with four-rowed stalks are those of Bower (1915, 1923, 1926). Holttum and Sen (1961), in their paper "Morphology and classification of the tree ferns" did not make a detailed examination of the sporangia, but based their comments mostly on Bower's publications. For a clear understanding of the structure of the sporangia with four-rowed stalks their ontogeny needs to be studied in detail.

Because it is readily available in cultivation in southern California, *Sphaeropteris cooperi* (F. Muell.) R.M. Tryon [*Cyathea cooperi* (F. Muell.) Domin] was chosen for study to serve as a model for the pattern of development of sporangia with four-rowed stalks.

Materials and Methods

The material used in this study was collected from plants in cultivation in Los Angeles, California. A specimen of this fern has been deposited in the herbarium of Rancho Santa Ana Botanic Garden (*Wilson 2067,* RSA). Slides are deposited at Rancho Santa Ana Botanic Garden.

Fertile pinnae of *Sphaeropteris cooperi* in early and increasingly mature stages of development were preserved in formalin-aceto alcohol (FAA). Sori were processed by three different methods: 1) Fertile pinnules were infiltrated with the tertiary butyl alcohol series, embedded in paraffin, and sectioned at 10μm. The sections were then stained in the Sharman (1943) series. 2) Fertile pinnules were cleared in 5% NaOH, bleached in 50% chlorine bleach, and stained in 3% tannic acid in 50% alcohol and 3% ferric chloride in 50%

alcohol (the alcoholic stains were used to prevent maceration). After dehydration in alcohol, the sori were dissected from the pinnule lamina and placed on a slide in Diaphane. The sori were then teased to separate the sporangia and a coverslip was mounted. This technique resulted in an enormous amount of damage, but methodical searches of the slides and a large number of dissections revealed undamaged sporangia. This procedure of clearing and staining the sporangia allows both sides of each developing sporangium to be studied. 3) Young cleared fertile pinnules stained in tannic acid and ferric chloride were imbedded in paraffin, sectioned at 20μm, and mounted on slides. These preparations were studied to confirm the early division patterns observed in the other preparations.

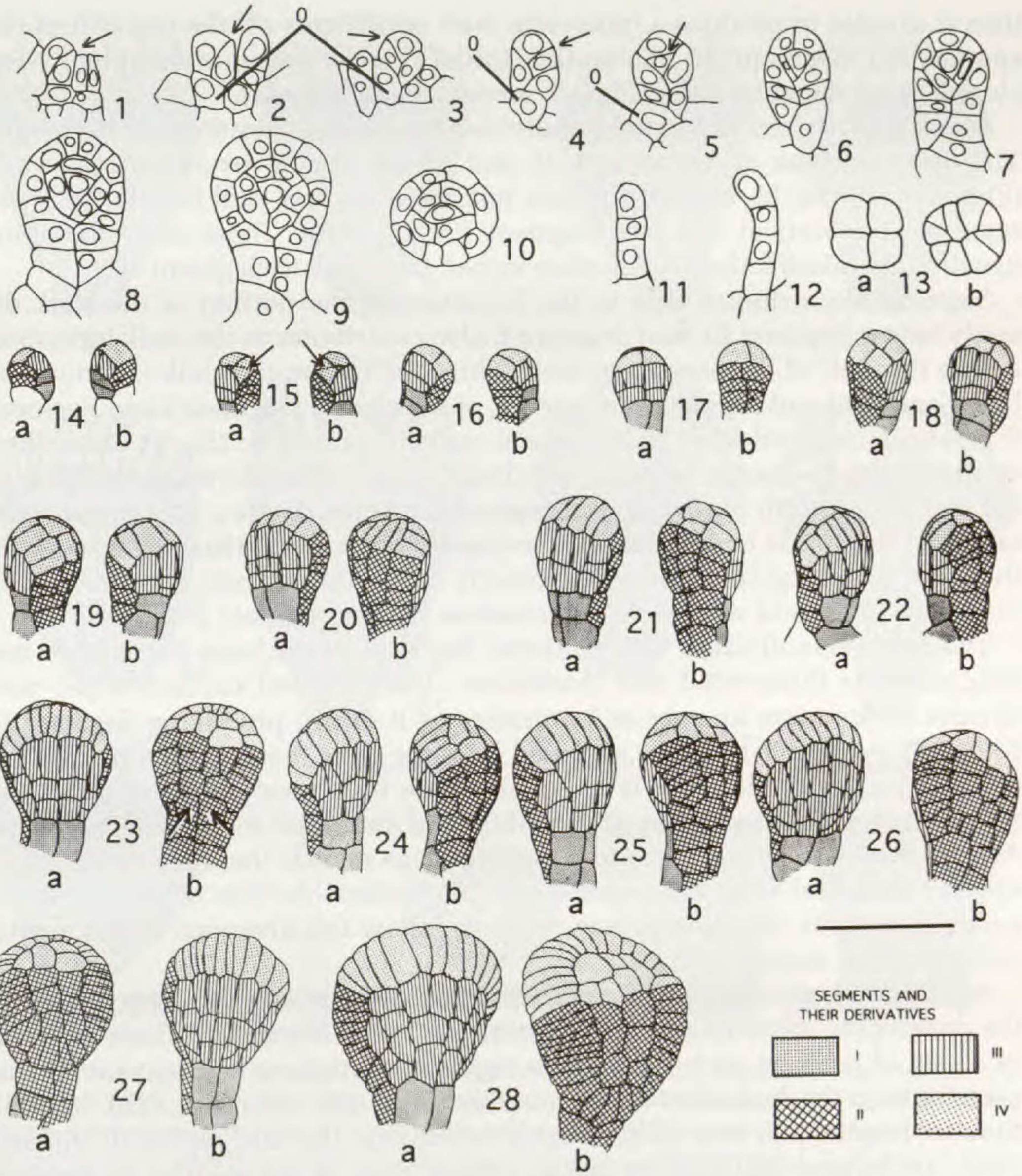
Mature sporangia were studied mounted in Crystal/Mount (Biomedia Corp., Foster City, California) on temporary slides. This process reduced the dehiscence of the capsules and at the same time prevented movement of the sporangia while being examined.

All illustrations were made with the aid of a Leitz drawing tube on a Leitz microscope. Both sides of each cleared sporangium were drawn and each sporangial "Segment" is shaded to facilitate comprehension of the cell lineages in the developing sporangium. The shading patterns used conform with those used in earlier sporangial ontogenetic studies in order to allow for easier comparisons.

Results

Sporangia of *Sphaeropteris cooperi* begin their development by the swelling of a single superficial cell of the receptacle, which soon becomes segmented by a diagonal wall that extends from approximately the surface of the receptacle, or a little above it, toward the base of the cell (Fig. 1, arrow). This first-formed wall segments the sporangial initial into a terminal cell, the "mother initial," and a basal cell, Segment 0. Segment 0 is found at the level of the receptacular superficial cells and takes no further part in the formation of the sporangium. Sporangia teased from the receptacle rarely show any evidence of the presence of Segment 0 and, therefore this segment can be identified clearly only in sectioned preparations (Figs. 1–5).

The cell called the mother initial divides to produce three segments that contribute to the formation of the stalk and capsule. Segment I is formed by a wall approximately perpendicular to that producing Segment 0 and intersecting this wall so as to divide the basal portion of the initial into two roughly equal halves (Fig. 2. arrow). The formation of Segment I is followed by a division of the mother initial that produces a wall roughly perpendicular to that of Segment I and parallel to the first-formed wall, giving rise to Segment II (Fig. 3, arrow). At this stage, the mother initial is 2-sided and wedge-shaped at the base. Segment III forms from a division more or less at a right angle to that of Segment II, directly above Segment I and parallel to it (Figs. 4, arrow; 14a, b). Although this series of divisions suggests the activity of an apical cell, the divisions of the mother initial always produce Segments I, II, and III, and



FIGS. 1–28. Sporangial ontogeny in *Sphaeropteris cooperi*. 1–10) Internal segmentation. All figures drawn from sectioned material. 1) Formation of Segment 0. 2) Formation of Segment I. 3) Formation of Segment II. 4) Formation of Segment III. 5) Formation of Segment IV. 6–9) Intercalary divisions of the Segments and formation of tapetal initials. 10) Cross section of young capsule showing tapetal initials and enclosed mother initial. 11, 12) Developing paraphyses. 13) Cross section of stalk; a = section of lower portion; b = section directly below capsule. 14–28) Superficial segmentation, earlier stages. All figures from cleared, stained whole mounts. Both sides of each sporangial primordium are illustrated and are designated "a" and "b." Arrows point to newly formed walls or cells. 0 = Segment 0. Scale bar = 10 μm.

then it divides to produce a transverse wall which cuts off the cap cell of the sporangium, Segment IV. The mother initial thereby becomes completely enclosed by its daughter cells (Figs. 5, arrow; 15a, b, arrows).

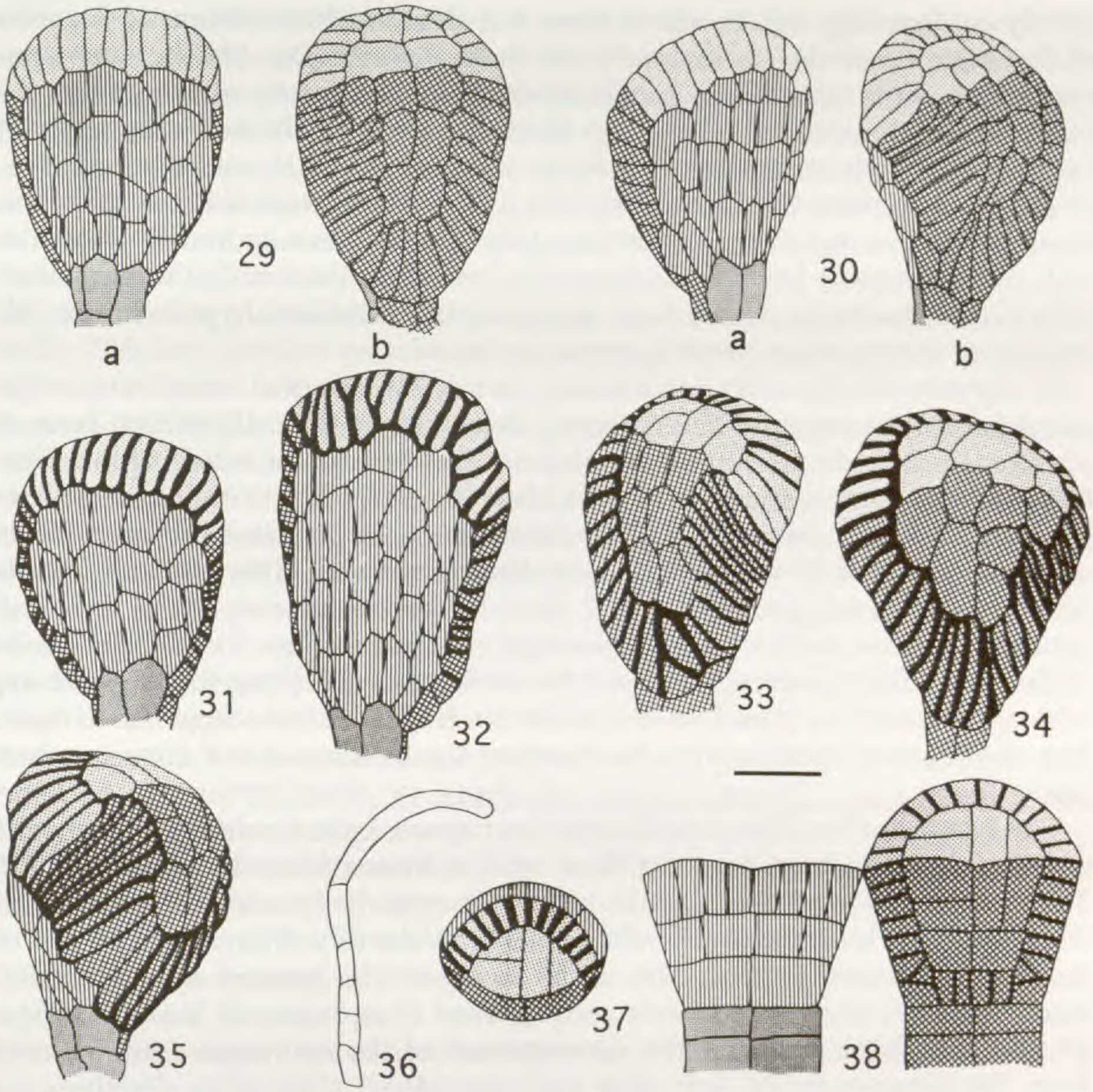
Segments I, II, and III become subdivided by a series of intercalary divisions. The first divisions of Segments I, II, and III are usually by horizontal walls (Figs. 14a, b; 15a, b), although in rare instances the first wall to subdivide the segment is a vertical one (see Segment III, Fig. 14b). These early divisions usually take place before the mother initial gives rise to Segment IV.

Segment I contributes only to the formation of the portion of the stalk directly below Segment III, and Segment II also contributes to the stalk formation and to the wall of the capsule as well. Thus, the sporangial stalk is formed by both Segment I and the proximal portion of Segment II. Segment I and Segment II first become subdivided by horizontal walls (Figs. 14a, b; 15a, b). Soon after, as the young sporangia enlarge, anticlinal, longitudinal divisions in each of the cells function in bisecting each segment and give rise to a sporangial stalk, which at its base is composed of four rows of cells (Figs. 13a, 14b, 17a, b). In the more distal region of the stalk, directly below the capsule, additional subdivisions associated with capsule formation become evident (Fig. 13b).

The transverse division that produces Segment IV encloses the mother initial, which is three-sided and cymbiform. The enclosed mother initial now divides in the same manner and sequence as it did in producing Segment II, III and IV, so that it becomes enclosed by three cells that separate it from the cells of the sporangial wall (Figs. 6–8). These innermost daughter cells give rise to the tapetum by means of periclinal and anticlinal divisions (Figs. 8, 9). A cross section through the capsule at this stage reveals that the enclosed cell appears elliptical (Fig. 10), whereas in longitudinal section it appears triangular (Figs. 8, 9). No attempt was made to follow the divisions of the tapetal cells or of the central cell.

Additional horizontal and vertical divisions accompany the enlargement of the developing sporangium. In the distal portion of Segment II these give rise to a row of cells on each side of the segment contiguous to Segment III that contribute to the formation of the annulus (Figs. 20b, 21b, 22b, 23b). In addition, in Segment II, two cells, roughly occupying the mid-region of the segment, are formed that link the lateral vertical rows of the annulus in Segment II and complete the U-shaped line of cells that produce the lower arc of the annulus (Fig. 23b arrows).

A series of divisions in Segment IV gives rise to the cells that complete the ring. The first subdivision of Segment IV is by a wall parallel to the one that produces Segment I (Fig. 16a, b). Divisions in each daughter cell perpendicular to this first wall complete the delimitation of the annular region in Segment IV (Fig. 18a). Additional divisions in the cells next to Segment III produce a row of cells that are contiguous to the distal portion of Segment III (Fig. 19 a,b). These cells become continuous with the annular cells of Segment II (Figs. 20b, 21b). The U-shaped row of cells in Segment II together with the row of cells in Segment IV produce the uninterrupted ring of cells that is the annulus (Figs. 27a, 28b). The annulus, therefore, always borders Segment III along its



FIGS. 29–38. Sporangial ontogeny in *Sphaeropteris cooperi*. 29, 30) Superficial segmentation, later stages. 31, 32) Mature sporangia, proximal faces. 33, 34) Mature sporangia, distal faces. 35) Mature sporangium, side view. 36) Mature paraphysis. 37–38) Diagrammatic analysis of segment derivatives in the mature sporangia. 37) Top view, Segment IV. 38) Segments I, II, III, and IV. Scale bar = 10 μm.

distal margin and each side; thus, Segment III becomes surrounded by the annulus except at its base where it abuts Segment I.

A group of cells of the annulus, either on the left side or the right side of Segment II and usually in contact with the ring cells of Segment IV, become subdivided by vertical walls into thin long cells that can be distinguished from the other cells of the annulus (Figs. 28b, 30b). Mature sporangia have 4–8 (usually 6) cells in this, the stomial region. There is, however, considerable variation in the thickening of the cells of the annulus, and the stomial cells are not always clearly differentiated. In some sporangia, the cell walls of all of the cells of the annulus become thickened and the stomial region is not

clearly evident (Fig. 34). In others there is a clearer differentiation of the cells of this region and the stomial cells are more distinct (Fig. 33). In some sporangia, the walls of the 2 or 3 cells of the annulus directly above and/or directly below the stomial cells either do not thicken or thicken only slightly. In these sporangia an epistomium forms when the cells above the stomial region do not become thickened (Fig. 33), and a hypostomium develops when those below the region do not thicken (Fig. 35). In some sporangia all of the cells of the annulus become thickened but in others thin-walled unthickened cells border the stomium and form an epistomium and/or a hypostomium. All manner of intermediate conditions can be found.

In Segment IV the cells not forming part of the annulus contribute to the distal face of the capsule. The divisions that produce the cells of both faces of the capsule cells do not follow any definite pattern and the resulting cell configuration in mature capsules is variable, consisting of 4–7 cells. In mature sporangia, it is not always possible to determine with precision the exact limits of Segments II and IV where they meet. Distortions and shifts of the cell walls occur as the sporangia enlarge and obscure the boundaries of the two segments. Therefore, in the mature sporangia illustrated (Figs. 31–35), the limits of the areas distinguishing Segment II and Segment IV along their border are only approximations based on the understanding of earlier ontogenetic stages. Any discrepancy is believed to be of minor significance and of not more than one cell.

The lateral walls of the capsule and the capsule base are derived from cells of Segment II and from Segment III, as well as from a few cells of Segment IV. The proximal face of the capsule is formed entirely by cells of Segment III (Figs.31, 32). The number of cells varies considerably, from about 15–28 or more, as does their pattern. This is the face with the greatest area; the distal face is smaller, made up of fewer cells derived from Segments II and IV (Figs. 33, 34). The later stages in the development of the sporangia result mostly from the increase in its size, with only occasional intercalary divisions increasing the number of cells. Subdivision of the cells of the annulus as the capsule enlarges, however, is not infrequently evident (Figs. 32–34).

Once the sporangia are fully enlarged and the spores are formed, the cells of the annulus become thickened (Figs. 31–35). All or most of the cells of the annulus develop a pronounced thickening and the mature sporangia possess an oblique uninterrupted bow.

Paraphyses are commonly found intermixed with the sporangia. These are initiated by the formation of a transverse wall in a superficial cell of the receptacle at the level of the surface of the receptacular cells. Additional transverse walls are produced that result in the formation of uniseriate filamentous paraphyses (Figs. 11,12, 36).

Discussion

The sporangium with a 4-rowed stalk, as described in *Sphaeropteris cooperi,* has a distinctly different developmental sequence from that known in the high-

er leptosporangiate ferns with 1- to 3-rowed stalks. Although both types originate from a single superficial cell of the receptacle that becomes divided into 5 basic structural segments, the organization and the subdivision of each of the segments does not produce the same sporangial structures.

In *Sphaeropteris,* the first division of the sporangial initial is by an oblique wall that extends from the level of the surface of the cells of the receptacle to below it. This division produces Segment 0, the lower of the two daughter cells, located at the level of the surface receptacular cells. Segment 0 does not become further subdivided and does not contribute to the formation of the stalk. This first division pattern contrasts sharply with that found in the higher leptosporangiate ferns, in which the location and orientation of the first-formed cell wall is critical in establishing the number of rows of cells in the stalk, and in which Segment 0 is an important structural component of the stalk (Wilson, 1958a, b). The one-rowed stalk results from the horizontal orientation of the first wall that is placed well above the level of the surface of the receptacular cells. In the two-rowed stalk and the three-rowed stalk the first wall is oblique, also above the level of the cells of the receptacle, and the number of rows in the stalk depends also on the orientation of the wall that produces Segment I (Wilson, 1958a). With rare exceptions, the walls intercalated in the segments or portions of the segments giving rise to the 1-, 2- and 3-rowed stalk are transverse, never longitudinal. An unusual variation was reported in *Anarthropteris,* in which the stalk is basically single-rowed at the base, but becomes complicated by various intercalated longitudinal and oblique divisions to become irregular, and thus, at different levels it varies from one cell to three cells or perhaps even more (Wilson, 1960).

The formation of the 4-rowed stalk of *Sphaeropteris* follows a different pattern. After the formation of Segment 0, the base of the sporangial initial becomes divided into two parts by Segments I and II. Each of these segments, usually after one or two transverse intercalary divisions, becomes bisected by longitudinal radial walls. In this way, the stalk base becomes 4-rowed.

Bower (1928), in describing the segmentation of the sporangium of *Hemitelia capensis* (L. f.) Sm. (= *Alsophila capensis* (L. f.) J. Sm., as treated by Conant, 1983: 366), wrote that, "The parent cell has a wedge-shaped base, and the first segment-wall is inserted on one of the oblique walls," and that "Further segmentation is by alternate cleavages in two rows, which are succeeded by the formation of a cap-cell." It appears that Bower identified the "first segment-wall" as the one that forms Segment I, rather that the one forming Segment 0. Segment 0 is difficult to see, but it is a constant component of the sporangia of *Sphaeropteris,* and probably also of *Hemitelia,* although it is obscured by its placement at the very base of the sporangial stalk.

If, in describing "alternate cleavages in two rows," Bower was suggesting the activity of an apical cell, my investigations do not support his view. In discussions pointing out that the sporangial stalk of the higher leptosporangiate ferns is not produced by the activity of an apical cell, I have described that it results from cells intercalated in the first segments of the sporangial primordium (Wilson, 1958a, b). This is also the pattern in *Sphaeropteris,* in

which the structure of the stalk is determined by the formation of Segments I and II and also by cells intercalated in these segments. The 2-rowed or alternate segmentation of *Sphaeropteris,* in which the walls of Segments 0–III are laid down one above the other and parallel to each other, gives rise to a 2-rowed stalk that becomes subdivided into 4 rows. Capsule formation in the higher leptosporangiate ferns is triradiate in that Segments I, II and III each contribute to one-third of its structure and to the subtending stalk. In *Sphaeropteris,* the capsule, when viewed in cross section, is bifacial and formed from 2 segments, but in higher leptosporantiates it is trifacial and formed from 3 segments. This is reflected also in the shape of the mother initial which, when enclosed, is cymbiform in *Sphaeropteris* and tetrahedral in higher leptosporangiates. Bower was correct, however, in describing the divisions that produce Segments I–IV, even though he was not aware of this segmentation pattern. The mother initial, after Segments I and II are formed, is indeed wedge-shaped, and Segments 0–III are formed above each other, as in two rows. Bower was also correct in describing the four-rowed stalk as resulting from, "Each of the segmental cells having been divided again by a radial wall."

In *Sphaeropteris* the portion of the stalk subtending the capsule develops from Segments I and II. Although initially 4-rowed, the stalk at the very base of the capsule becomes further subdivided by additional walls as the sporangium develops to become more elaborate (see Fig. 13b). In the higher leptosporangiate ferns, the stalk directly below the capsule is always 3-rowed and is derived from Segments I, II, and III (Wilson, 1958a). Bower (1923), in a comparison of the sporangial stalk of various ferns, correctly suggested that the difference in stalk structure resulted from the pattern of segmentation of the sporangial initial. It is the orientation of the walls that form these segments that is critical in determining the number of rows in the sporangial stalk.

In *Sphaeropteris* the capsule is derived from Segments II and III, which contribute to the lower lateral faces, and Segment IV, which forms the cap and part of the upper portion of the distal face (see Fig. 37). This again reflects the two-rowed pattern of segmentation of the sporangial initial. In the higher leptosporangiate ferns, the capsule forms from Segments I, II, and III, which contribute to the lower lateral faces, and also from Segment IV, which contributes to the upper portion of both the distal and proximal faces. This structural difference, described by Bower as "triradiate," results from the different patterns of segmentation of the sporangial initial.

The annulus in *Sphaeropteris* develops from Segments II and IV and forms a continuous oblique ring. This contrasts with that in the higher leptosporangiate ferns, in which the annulus develops from Segments II, III, and IV and is vertical and interrupted by the stalk. The stomial region in *Sphaeropteris* forms in Segment II either on the left or the right side of the segment. These cells tend to be thinner and longer than the other cells of the annulus and the number of cells is variable, as is their thickening. Also, thin-walled epistomial or hypostomial cells may or may not become evident. The stomium in the higher leptosporangiate ferns is more precisely defined in that a pair of stomial cells and an epistomium and hypostomium are usually well differentiated.

The stalk of the sporangia of *Sphaeropteris* is short, rarely more that two cells tall; the sporangial stalks of *Sphaeropteris* do not undergo any significant elongation. Increase in cell size takes place uniformly in all cells of the sporangia as they mature.

The differences seen in the ontogeny and structure of the sporangia of *Sphaeropteris* are significant. There are at least two types of leptosporangia. Bower (1923) believed that there was no, "General or necessary relation between initial segmentation and the production of mature structures." This is clearly not the case. Similar appearing structures, such as the annulus, have different origins and should be compared to each other with caution. It is not possible for me to speculate on the origin of either pattern of development without additional studies of other fern sporangia, especially in basal groups such as the Gleicheniaceae, Osmundaceae, Plagiogyraceae, Schizaeaceae, etc.

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